

Figure 1 (A-F)

Construct Forms Comprising at Least one Single-Stranded Region

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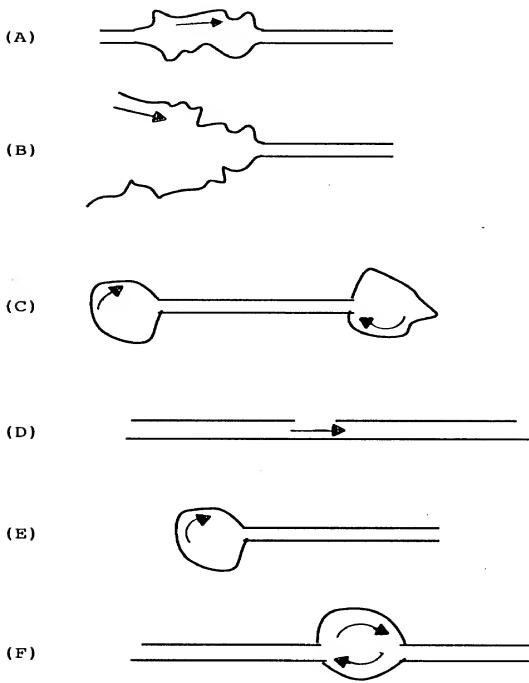


Figure 2 (A-F)

Functional Forms of the Construct

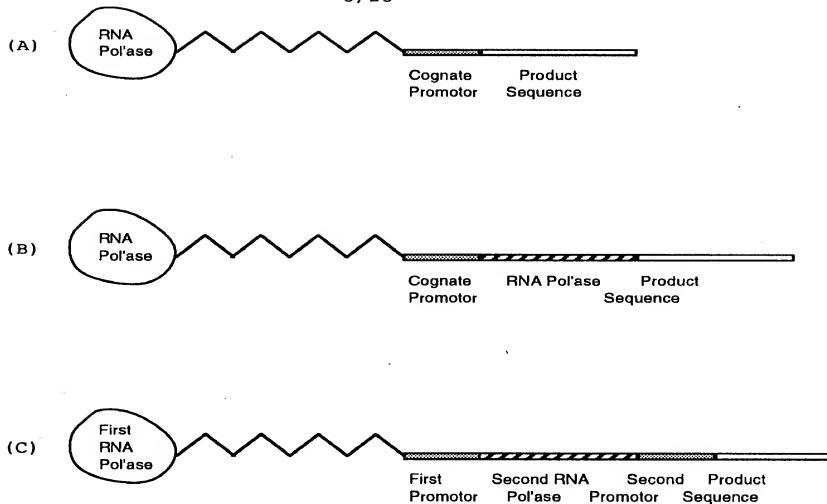


Figure 3 (A-C)

**Three Constructs with an RNA Polymerase
Covalently Attached to a Transcribing Cassette**

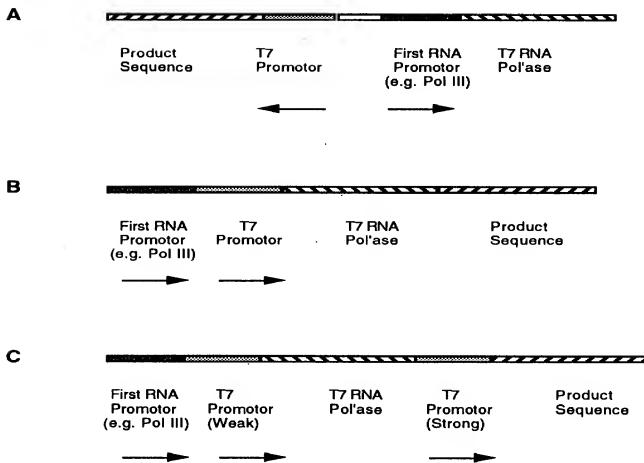


Figure 4 (A-C)

**Three Constructs with Promoters
for Endogenous RNA Polymerase**

M13mp18. Seq Length: 7250

1. AATGCTACTA CTATTAGTAG AATTGATGOC ACCTTTTCAG CTOGOGCCCC
 51. AAATGAAAAT ATAGCTAAAC AGGTTATTGA CCATITGOGA AATGTATCTA
 101. ATGGTCAAAC TAAATCTACT CGITTOGCAGA ATTGGGAATC AACTGTTACA
 151. TGGAATGAAA CTTOCAGACA CGGTACTTTA GTTGCAATT TAAAACATGT
 201. TGAGGCTACAG CACCAGATTG AGCAATTAAAG CTCTAAGOCA TOCGAAAAAA
 251. TGACCTCTTA TCAAAAGGAG CAATTAAAGG TACTCTCTAA TOCTGACCTG
 301. TTGGAGTTTG CTTCOOGGTCT GGTTGCGTTT GAAGCTOGAA TAAAACGOG
 351. ATATTGAAAG TCTTTGCGGC TTCCCTCTTAA TCTTTTTGAT GCAATCGCT
 401. TTGCTTCTGA CTATAATAGT CAGGGTAAAG ACCTGATTT TGATTTATGG
 451. TCATTCTCGT TTCTGAACT GTTAAAGCA TTGAGGGGG ATTCAATGAA
 501. TATTTATGAC GATTGCGAG TATTGGACGC TATCCAGTCT AACCATTTA
 551. CTATTACCCC CTCTGGCAAA ACTTCTTTG CAAAGGCTC TOGCTATTTT
 601. GGTTTTTATC GTGCGTCTGGT AAAAGGCGT TATGATAGTG TTGCTCTTAC
 651. TATGCGCTCGT AATTCCCTTT GGGCGTTATGT ATCTGCATTA GTGAAATGTT
 701. GTATTCCTAA ATCTCAACTG ATGAATCTTT CTACCTGAA TAATGTTGTT
 751. CGGTTAGTTTC GTTTTAACTT CGTAGATTT TCTTCCCAAC GTGCTGACTG
 801. GTATAATGAG CCAGTTCTTA AAATOGCATA AGGTAATTCA CAATGATTAA
 851. AGTTGAAATT AAACCATCTC AAGGCCAATT TACTACTCGT TCTGGTGTTC
 901. TGGTCAGGGC AAGCTTATT CACTGAATGA GCAGCTTGT TACGTTGATT
 951. TGGGTAATGA ATATCGGGT CTTGCGAAG ATTACTCTTG ATGAAGGTCA
 1001 GCGAGGCTAT GCGCGCTGGTC TGACACCGT TCATCTGTCC TCTTTCAAAG
 1051 TTGGTCAGTT CGGGTTCCTT ATGATTGACC GTCTCGGCTC CGTTCCGGCT
 1101 AAGTAACATG GAGCAGGTOG CGGATTGOGA CACAATTAT CAGGOGATGA
 1151 TACAAATCTC CGTTGTCACCTT TGTTTGGCGC TTGGTATAAT CGCTGGGGGT
 1201 CAAAGATGAG TGTTTTAGTG TATTCTTTCG CCTCTTTCGT TTAGGTTGG

Figure 5

M13mp18 Nucleic Acid Sequence

1251	TGCGTTTGTAA	GTCGGCATTAC	GTATTTTACCC	CCTTTAATGG	AAACTTCTCTC
1301	ATGAAAAAGT	CTTTAGTCTT	CAAAGGCTCT	GTAGCGGTTG	CTACCCCTGTT
1351	TCGATGCTG	TCTTTGCTG	CTGAGGGTGA	CGATCCCCCA	AAAGGGGCGT
1401	TTAACCTOCT	GCAAGGCTCA	GCGACCGAAT	ATATCGGTTA	TGCGTGGGGCG
1451	ATGGTTGTTG	TCATTTGCTG	CGCAACTATC	GGTATCAAGC	TGTTTAAGAA
1501	ATTCACCTCG	AAAGCAAGCT	GATAAACCGA	TACAATTAAA	GCCTCCCTTT
1551	GGAGCCTTTT	TTTTGGAGA	TTTCAACGT	GAAAAAAATTA	TTATTGCAAA
1601	TTCCCTTAGT	TGTTCCCTTC	TATTCTCACT	CGCGTAAAC	TGTTGAAAGT
1651	TGTTTAGCAA	AACCCATAC	AGAAAATTCA	TTTACTAACG	TCTGGAAAGA
1701	CGACAAAAC	TTAGATCGTT	ACGCTAACTA	TGAGGGTTGT	CTGTGGAATG
1751	CTACAGGGT	TGTAGTTGT	ACTGGTGACG	AAACTCAGT	TTACGGTACA
1801	TGGGTCTCTA	TTGGGCTTGC	TATCCCTGAA	AATGAGGGTG	GTGGCTCTGA
1851	GGGTGGGGT	TCTGAGGGTG	GGGGTTCTGA	GGGTGGGGT	ACTAAACCTC
1901	CTGAGTAACGG	TGATACACCT	ATTCGGGCT	ATACTTATAT	CAACCCCTCTC
1951	GACGGCACCT	ATOCGGCTGG	TACTGAGCAA	AACCGCTA	ATCCTAATCC
2001	TTCTCTTGAG	GAGTCTCAGC	CTCTTAATAC	TTTCATGTTT	CAGAATAATA
2051	GGTTCOGAA	TAGGCAGGGG	GCATTAACGT	TTTATAACGC	CACTGTTACT
2101	CAAGGCACTG	ACCCCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2151	AAAAGOCATG	TATGACGCC	ACTGGAAACG	TAAATTCAA	GAATGGCTT
2201	CAAGGCACTG	ACCCCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2151	AAAAGOCATG	TGCGTCAACC	TCTGTCAAT	GCTGGGGCGG	GCTCTGGTGG
2201	TOCATTCTGG	CTTTAATCAA	GATCCATTG	TTTGTGAATA	TCAAGGCCAA
2251	TGCGTCTGACC	TGCGTCAACC	TCTGTCAAT	GCTGGGGCGG	GCTCTGGTGG
2301	TGGTTCTGGT	GGGGGCTCTG	AGGGTCTG	CTCTGAGGGT	GGGGGTTCTG
2351	AGGGTCTGGG	CTCTGAGGGG	GGGGGTTGCG	GTCGTGGCTC	TGGTTCOGGT
2401	GATTTTGATT	ATGAAAAGAT	GGCAAAAGCT	AATAAGGGGG	CTATGACCGA
2451	AAATGCGAT	AAAAACGCGC	TACAGTCTGA	CGCTAAAGGC	AAACTTGATT

Figure 5**M13mp18 Nucleic Acid Sequence**

2501	CTGTOGCTAC	TGATTAOGGT	GCTGCTATOG	ATGGTTTCAT	TGGTGAOGTT
2551	TOGGGCCTTG	CTAATGGTAA	TGGTGCTACT	GGTGATTITG	CTGGCTCTAA
2601	TTCCCAAATG	GCTCAAGTOG	GTGACGGITGA	TAATTCAACCT	TTAACATAATA
2651	ATTTCCGTC	ATATTTACCT	TOCGTCACTC	AATOGGTTGA	ATGTOGCGCT
2701	TTTGTCTTTA	GGCGTGGTAA	ACCATATGAA	TTTTCTATTG	ATTGTGACAA
2751	AATAAACCTA	TTCGTTGTTG	TCTTTCCGTT	TCTTTTATAT	GTGCGCAACT
2801	TTATGTATGT	ATTTTCTACCG	TTTGCTAACAA	TACTGCGTAA	TAAGGAGTCT
2851	TTATCATGCC	AGTTCTTITG	GGTATTCCGT	TATTATTGCG	TTTCCTCGGT
2901	TTCCCTCTGG	TAACCTTGTG	GGGCTATCTG	CTTACTTTTC	TTAAAAAGGG
2951	CTTCGGTAAG	ATAGCTATTG	CTATTTCAT	GTTTCTTGCT	CTTATTATTG
3001	GGCTTAACTC	AATTCTGTG	GGTTATCTCT	CTGATATTAG	CGCTCAATT
3051	CCCTCTGACT	TTGTTCAAGG	TGTTCAAGTA	ATTCTCCCGT	CTAATGCGCT
3101	TCCCTGTTTT	TATGTTATT	TCTCTGTAAA	GGCTGCTATT	TTCATTTTGT
3151	ACGTTAAACA	AAAAATCGTT	TCTTATTTGG	ATTGGGATAA	ATAATATGGC
3201	TGTTTATTTT	GTAACTGGCA	AATTAGGCTC	TGGAAAGACG	CTGGTTAGCG
3251	TTGGTAAGAT	TCAGGATAAA	ATTGTAAGCTG	GGTGCAAAAT	AGCAACTAAT
3301	CTTGATTAA	GGCTTCAAA	OCTCOOGCA	GTGGGGAGGT	TGCTAAAC
3351	GGCTCGCGTT	CTTAGAACAC	OGGATAAGGC	TTCTATATCT	GATTGCTTG
3401	CTATTTGGCG	CGGTAATGAT	TOCTACCGAATG	AAAATAAAAA	CGGCTTGCTT
3451	GTTCTOGATG	AGTGCGGTAC	TTGGTTTAAT	ACCGGTTCTT	GGAATGATAA
3501	GGAAAGACAG	CGGATTATTG	ATTGGTTCT	ACTGCTCGT	AAATTAGGAT
3551	GGGATATTAT	TTTCTTGTG	CAGGACTTAT	CTATTGTTGA	TAAACAGGCG
3601	CGTTCTGCAT	TAGCTGAACA	TGTTGTTTAT	TGTCGTCGTC	TGGACAGAAAT
3651	TACTTTACCT	TTTGTGGTAA	CTTTATATTC	TCTTATTACT	GGCTCGAAA
3701	TGCGCTCTGAC	TAAATTACAT	GTGGGCGTIG	TTAAATATGG	CGATTCTCAA
3751	TTAAGCGCTA	CTGTTGAGCG	TTGGCTTTAT	ACTGGTAAGA	ATTTGTATAA
3801	CGCATATGAT	ACTAACACAGG	CTTTTCTAG	TAATTATGAT	TOCGGTGTTT

Figure 5**M13mp18 Nucleic Acid Sequence**

3851	ATTCTTATT	AAAGCCTTAT	TTATCACAOG	GTCGGTATT	CAAACCATTA
3901	AATTTAGGTC	AGAAGATGAA	ATTAAC TAAA	ATAATATTGA	AAAAGTTTC
3951	TGCGGTTCTT	TGTCCTTGCGA	TTGGAA TTGC	ATCAGCATT	ACATATAGTT
4001	ATATAACCCA	ACCTAACGCG	GAGGTAA	AGGTAGTC	TCAGACCTAT
4051	GATTTTGATA	AATTCACTAT	TGACTCTTCT	CAGCGTC	ATCTAAGCTA
4101	TCGCTATGTT	TTCAAGGATT	CTAAGGAAA	ATTAATTAA	AGCGACGATT
4151	TACAGAAGCA	AGGTTATTCA	CTCACATATA	TTGATTATG	TACTGTTCC
4201	ATAAAAAAAG	GTAATTCAA	TGAAATTGTT	AAATGTAATT	AATTTGTTT
4251	TCTTGATGTT	TGTTTCATCA	TCTTC	CTCAGGTAAT	TGAAATGAA
4301	AATTGCGTC	TGCGCGATT	TGTAAC	TATTCAAAGC	AATCAGGOGA
4351	AATCCGTTATT	GTTTCTCGG	ATGAAAAGG	TACTGTTACT	GTATATTCA
4401	CTGACGTTAA	ACCTGAAAAT	CTACGCAATT	TCTTATTTC	TGTTTACG
4451	GCTAATAATT	TTGATAATGGT	TGGTTCAATT	CC	TTCAGAAGTA
4501	TAATOCAAAC	AATCAGGATT	ATATTGATGA	ATTGOCATCA	TCTGATAATC
4551	AGGAATATGA	TGATAATT	GGCTCCTCTG	GTGGTTCTT	TGTTGCGAA
4601	AATGATAATG	TTACTCAAC	TTTAAAATT	AATAACGTT	GGCCAAAGGA
4651	TTAATACGA	GTTGTCGAAT	TGTTTGAAA	GTCTAAACT	TCTAAATCCT
4701	CAAATGTATT	ATCTATTGAC	GGCTCTAAC	TATTAGTTGT	TAGTGCTCT
4751	AAAGATATT	TAGATAACCT	TCCTCAATT	CTTCTACTG	TTGATTGCC
4801	AACTGACCAG	ATATTGATTG	AGGGTTGAT	ATTTGAGGTT	CAGCAAGGTG
4851	ATGCTT	TTTTCATTT	GCTGCTG	CTCAGCGTGG	CACTGTTGCA
4901	GGGGTGT	ATACTGACOG	CC	GT	CTGCTGGTGG
4951	TTGGTTGGT	ATTTTAATG	GCGATGTTT	AGGGCTATCA	GTTGCGGCAT
5001	TAAGACTAA	TAGCCATTCA	AAAATATTGT	CTGTGCGACOG	TATTCTTACG
5051	CTTTCAGGTC	AGAAGGGTC	TATCTCTGTT	GGCCAGAATG	TCCCTTTTAT
5101	TAAGACTAA	TAGCCATTCA	AAAATATTGT	CTGTGCGACOG	TATTCTTACG
5151	CGATTGAGOG	TCAAAATGT	GGTATT	TGAGCGTTT	TCCTGTTGCA

Figure 5

M13mp18 Nucleic Acid Sequence

5201	ATGGCTGGCG	GTAATATTGT	TCTGGATATT	ACAGCAAGG	CGATAGTTT
5251	GAGTTCTCT	ACTCAGGCAA	GTGATGTTAT	TACTAACAA	AGAAGTATIG
5301	CTACAAACGGT	TAATTTGCGT	GATGGACAGA	CTCTTTACT	GGTGAAGCTC
5351	ACTGATTATA	AAAACACTTC	TCAAGATTCT	GGGCTAOGT	TCCTGTCTAA
5401	AATCCCTTTA	ATGGGCCCTC	TGTTTAGCTC	CGCTCTGAT	TOCAAOGAGG
5451	AAACCAOGTT	ATACTGCTC	GTCAAAGCAA	CCATAGTACG	CGCCCTGTAG
5501	GGGOGCATTA	AGGGGGGGGG	GTGTTGGTGT	TAOGOGCAGC	GTGACOGCTA
5551	CACTTGCGAG	CGGCCCTAGCG	CGCGCTCCTT	TGCCTTTCTT	CCCTTCCCTT
5601	CTGGCGACGT	TOGGGGCTT	TOCGGCTCAA	GCTCTAAATC	GGGGGCGTOC
5651	TTTGGGTTTC	CGATTTAGTG	CTTTACGGCA	OCTOGACCCC	AAAAAAACTTG
5701	ATTTGGGTGA	TGGTTCAOGT	AGTGGGOCAT	CGOOGCTGATA	GAOGGTTTTT
5751	CGCCCTTGTG	CGTTGGAGTC	CACGTTCTTT	AATAGTGGAC	TCTTGTTCGA
5801	AACTGGAAACA	ACACTCAACC	CTATCTGGG	CTATTCTTTT	GATTITATAAG
5851	GGATTTTGCC	GATTTCGGAA	CGACCATCAA	ACAGGATTTT	CGCGTGTGG
5901	GGCAAACCGAG	CGTGGACCGC	TTGCTGCAAC	TCTCTCAGGG	CGAGGGGTG
5951	AAGGGCAATC	AGCTGTGGCC	CGTCTCGCTG	GTGAAAAGAA	AAACACCGT
6001	GGCGCCCAAT	AOGCAAACCG	CGCTCTCGG	CGCGTTGGGC	GATTCTAA
6051	TCACTCTGGC	AOGACAGGTT	TOOOGACTGG	AAAGCGGGCA	GTGAGOGCAA
6101	CGCAATTAAAT	GTGAGTTAGC	TCACTCATTA	GGCAACCCAG	GCTTACACT
6151	TTATGCTTCC	GGCTCGTATG	TTGTGTGGAA	TTGTGAGGGG	ATAACAATT
6201	CACACAGGAA	ACAGCTATGA	CCATGATTAC	GAATTGAGC	TOGGTACCOG
6251	GGGATCTCT	AGAGTOGACC	TGGCAGGCATG	CAAGCTTGGC	ACTGGCGTC
6301	GGTTTACAAC	GTGCGTACTG	GGAAAAACCT	GGCGTTAACCC	AACTTAATOG
6351	CCCTTGCGAGCA	CAATCCCCTT	TOGCCAGCTG	GGGTTAACGC	GAAGAGGCGC
6401	GCACCGATOG	CCCTTCCCAA	CAGTTGCGCA	GGCTGAATGG	CGAATGGCGC
6451	TTTGCGCTGGT	TTCOGGCAAC	AGAAGGGTGT	CGGAAAGCT	GGCTGGAGTG
6501	CGATCTTCT	GAGGGCGATA	CGGTGCGTGT	CGCGTAAAC	TGGCAGATGC

Figure 5

M13mp18 Nucleic Acid Sequence

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6551	AOGGTTAOGA	TGCGGCCATC	TACACCAAOG	TAACCTATCC	CATTACGGTC
6601	AAATCGGGT	TTGTTCCAC	GGAGAACGCG	ACGGGTTGTT	ACTCGCTCAC
6651	ATTTAATGTT	GATGAAAAGCT	GGCTACAGGA	AGGCGAGACG	CGAATTATTT
6701	TTGATGGGTT	TCCTATTGGT	TAACAAATGA	GCTGATTTAA	CAAAATTTA
6751	AOGCGAATTT	TAACAAAATA	TTAACGTTA	CAATTTAAAT	ATTTGCTTAT
6801	ACAATCTTC	TGTTTTGGG	GCTTTCTGA	TTATCAACCG	GGGTACATAT
6851	GATTGACATG	CTAGTTTAC	GATTACCGTT	CATCGATTCT	CTTGTTTGCT
6901	CCAGACTCTC	AGGCAATGAC	CTGATAGCCT	TTGTAGATCT	CTCAAAAATA
6951	GCTACOCTCT	CGGGCATGAA	TTTATCAGCT	AGAAOGGTTG	AATATCATAT
7001	TGATGGTGAT	TTGACTGTCT	CGGGCTTTC	TCACCCCTTT	GAATCTTAC
7051	CTACACATTA	CTCAGGCATT	GCATTTAAA	TATATGAGGG	TTCTAAAAAT
7101	TTTATCCCT	GGTTGAAAT	AAAGGCTTCT	CGCGAAAG	TATTACAGGG
7151	TCATAATGTT	TTTGGTACAA	CGGATTTAGC	TTTATGCTCT	GAGGCTTAT

Figure 5

M13mp18 Nucleic Acid Sequence

COMPLEMENTARY TO M13

POSITION	5' * * 3'	POSITION	
645	AGCAACACTATCATA	631	M 13/ 1
615	ACGACGATAAAAACC	601	M 13/ 2
585	TTTGCAAAAGAAGT	571	M 13/ 3
555	AATAGTAAATGTTT	541	M 13/ 4
525	CAATACTCGGAATG	511	M 13/ 5
495	TGAATCCOCCTCAA	481	M 13/ 6
465	AGAAAAGGAGAAATGA	451	M 13/ 7
435	CAGGTCTTACOCTG	421	M 13/ 8
405	AGGAAAGGCGATTGC	391	M 13/ 9
375	AGGAAGCCGAAAGA	361	M 13/10

COMPLEMENTARY TO SS PHAGE DNA

POSITION	5' * * 3'	POSITION	
351	ATATTTGAAGTCCTT	366	M 13/11
371	TCTTTTGATGCAAT	386	M 13/12
391	CTATAATCTCAGGG	406	M 13/13
411	TGATTTATGGTCATT	426	M 13/14
431	GTTTAAAGCATTTGA	446	M 13/15
451	TATTTATGAOGATTC	466	M 13/16
471	TATCCAGTCTAAACA	486	M 13/17
491	CTCTGGCAAAACTTC	506	M 13/18
511	TCGCTATTTGGTTT	526	M 13/19
531	AAACGAGGGTTATGA	546	M 13/20

Figure 6

Primers for Nucleic Acid Production
Derived from M13mp18 Sequence

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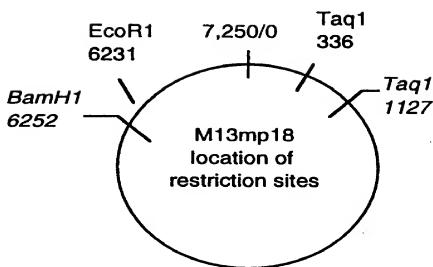


Figure 7

Appropriate M13mp18 Restriction Sites

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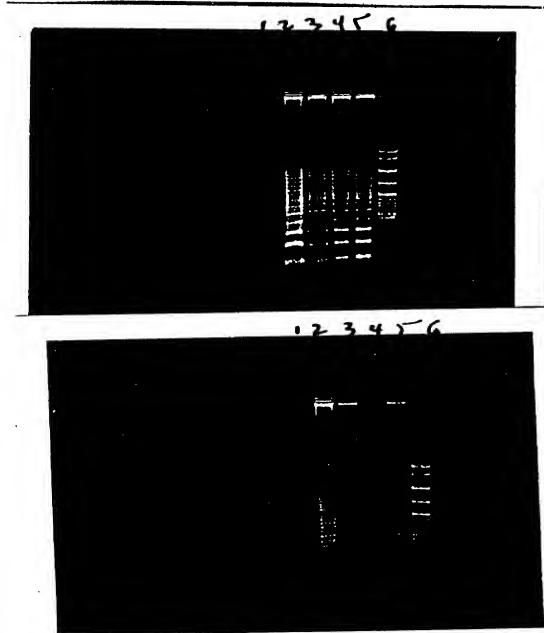
- Lane 1: from calf thymus + Taq digested mp18 amplification reaction
- Lane 2: from Taq digested mp18 amplification reaction
- Lane 3: from calf thymus amplification reaction
- Lane 4: øX174 Hinf1 size marker

Figure 8



- Lane 1: no template
- Lane 2: mp18 template, phosphate buffer
- Lane 3: Mspl/pBR322 size marker
- Lane 4: mp18 template, MOPS buffer

Figure 9



Top= (+) Template

Bottom= (-) Template

Lane 1: phosphate buffer

Lane 2: MES

Lane 3: MOPS

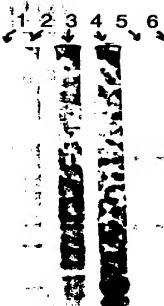
Lane 4: DMAB

Lane 5: DMG

Lane 6: pBR322/MspI size marker

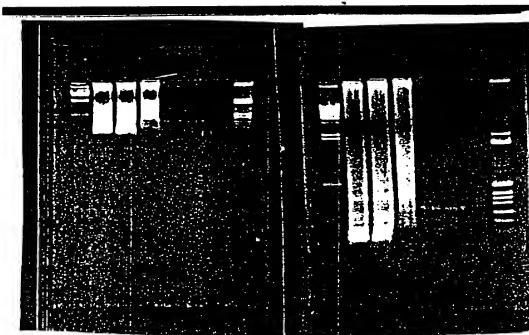
Figure 10

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- Lane 1: DMAB buffer, no template
- Lane 2: DMAB buffer, mp18 template
- Lane 3: DMG buffer, no template
- Lane 4: DMG buffer, mp18 template
- Lane 5: No reaction
- Lane 6: 200 ng Taq I digested mp18
size marker/positive control

Figure 11



First Time Interval Second Time Interval

Agarose Gel Analysis

- Lane 1: lambda Hind III marker
- Lane 2: Amp/Untreated
- Lane 3: Amp/Kinased
- Lane 4: Amp/Kinased/Ligated
- Lane 5: PCR/Untreated
- Lane 6: PCR/Kinased
- Lane 7: PCR/Kinased/Ligated
- Lane 8: øX174/Hinf1 marker

Figure 12

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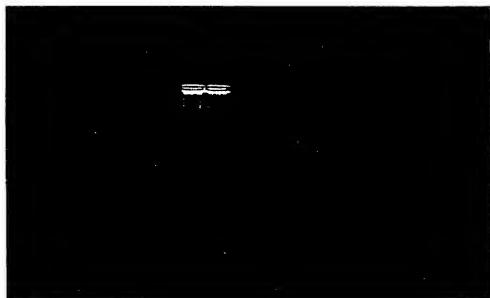


Figure 13

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1 2 3 4 5 6



- Lane 1: Primers alone
 - Lane 2: Primers + taq digested M13 DNA
 - Lane 3: Molecular weight markers
 - Lane 4: Primers + RNA
 - Lane 5: Primers alone
 - Lane 6: M13 digested DNA
- Buffer was dimethyl amino glycine, pH 8.6

Figure 14

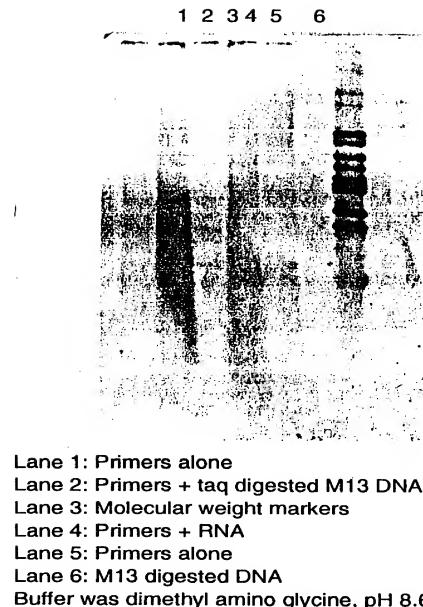


Figure 15

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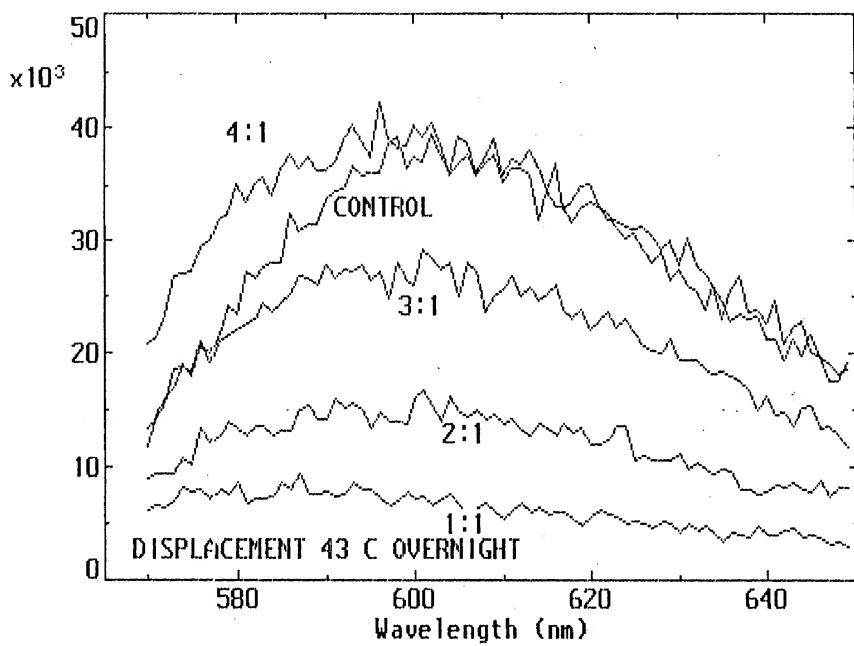


Figure 16

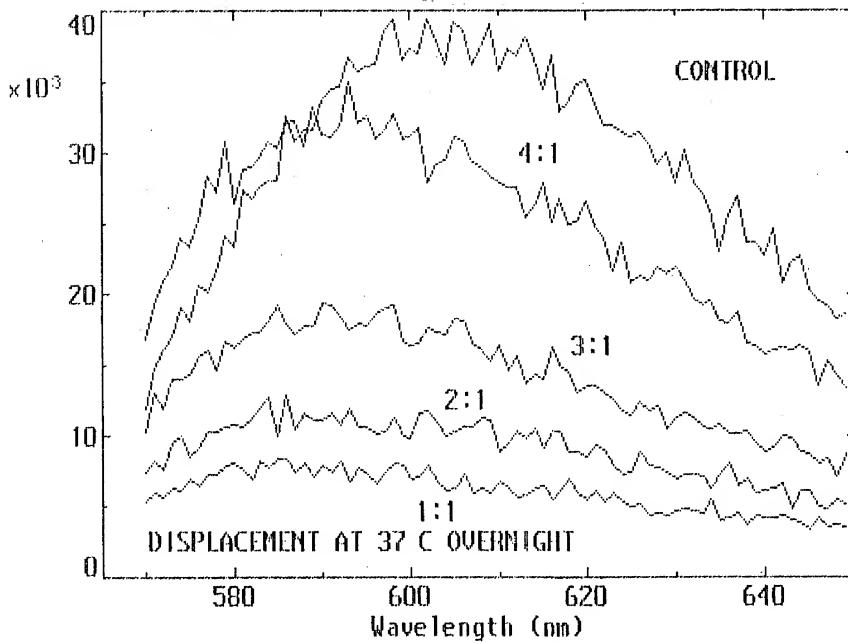


Figure 17

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pIBI 31-BH5-2

fmet AUG of Lac z (T7 Promotor region....
LAC PROMOTOR..ATG ACC ATG ATT ACG CCA GAT ATC AAA TTA ATA CGA CTC ACT ATA
oligo 50-mer 3'- tac t'aa t'gc ggt* ct'a t'ag t'Vt aat* tat* gct* gag t'ga t'at* c-5'
10 base insert

T7 RNA Start {«« T3 Promotor Region }
IGGG CTC ICCT TTA GTG ACG GTT AAT
...»} ← T3 Start Signal

pIBI 31 BSII/HCV

fmet AUG of Lac z (T3 Promotor region -») T3 RNA Start
LAC PROMOTOR ..ATG ACC ATG ATT ACG CCA AGC TCG AAA TTA ACC CTC ACT AAA /GGG
oligo 50-mer 3'- tac t'aa t'ac t'aa t'gc ggt* t'V--10 base insert--.....
(«- T7 Promotor Region)
MULTIPLE CLONING SITE + 390 BASE INSERT CTA /TAG TGA GTC CGT ATT AAT....
← T7 Start Signal
5'-ct'a t'ag t'ga gt*c gt*a tt*a at*.....

Figure 18